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Prevalence and Characterization of Shiga Toxin-Producing *Escherichia coli* in Swine Feces Recovered in the National Animal Health Monitoring System's Swine 2000 Study

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A study was conducted to determine the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) in swine feces in the United States as part of the National Animal Health Monitoring System's Swine 2000 study. Fecal samples collected from swine operations from 13 of the top 17 swine-producing states were tested for the presence of STEC. After enrichment of swine fecal samples in tryptic soy broth, the samples were tested for the presence of stx_1 and stx_2 by use of the TaqMan *E. coli* STX1 and STX2 PCR assays. Enrichments of samples positive for stx_1 and/or stx_2 were plated, and colony hybridization was performed using digoxigenin-labeled probes complementary to the stx_1 and stx_2 genes. Positive colonies were picked and confirmed by PCR for the presence of the stx_1, stx_2, or stx_{2e} genes, and the isolates were serotyped. Out of 687 fecal samples tested using the TaqMan assays, 70% (484 of 687) were positive for Shiga toxin genes, and 54% (370 of 687), 64% (436 of 687), and 38% (261 of 687) were positive for stx_1, stx_2, and both toxin genes, respectively. Out of 219 isolates that were characterized, 29 (13%) produced stx_1, 14 (6%) produced stx_2, and 176 (80%) produced stx_{2e}. Twenty-three fecal samples contained at least two STEC strains that had different serotypes but that had the same toxin genes or included a strain that possessed stx_1 in addition to a strain that possessed stx_2 or stx_{2e}. The STEC isolates belonged to various serogroups, including O2, O5, O7, O8, O9, OX10, O11, O15, OX18, O20, O57, O65, O68, O69, O78, O91, O96, O100, O101, O120, O121, O152, O159, O160, O163, and O untypeable. It is noteworthy that no isolates of serogroup O157 were recovered. Results of this study indicate that swine in the United States harbor STEC that can potentially cause human illness.

Shiga toxin-producing *Escherichia coli* (STEC), also known as verotoxin-producing *E. coli* (VTEC), comprises a serologically diverse group of pathogens that cause disease in humans and animals (4, 25, 27). Enterohemorrhagic *E. coli* O157:H7, the most prominent STEC, has caused numerous outbreaks of food-borne disease, hemorrhagic colitis, and hemolytic-uremic syndrome (HUS) worldwide (32, 39). However, over 200 non-O157 STEC serotypes have been identified, and over 100 strains have been associated with human illness (38, 51). In Australia, Argentina, and many European countries, infection with non-O157 STEC serotypes is common and may account for the majority of the cases of HUS (38). The common feature of STEC strains is the production of Shiga toxin 1 (Stx1) and/or Shiga toxin 2 (Stx2) or variants of Stx1 (Stx1c and Stx1d) or Stx2 (Stx2c, Stx2d, Stx2e, Stx2f, and Stx2g) (11, 20, 30, 46). The STEC strains that cause edema disease in swine produce the Stx2e variant (13). Strains harboring stx_{2e}, however, have also been isolated from patients with diarrhea and HUS (35, 44, 49).

A number of studies have demonstrated that animals, including cows, sheep, goats, and pigs, are reservoirs for different STEC strains, including serogroups that have been associated with human illness (3, 5, 12, 18, 23, 26). Botteldoorn and coworkers (10) isolated STEC that possessed the stx_{2e} variant gene from 56 of 177 (32%) pig rectal swabs and reported that 14 and 5% of these isolates were positive for the *eaeA* and *hlyA* virulence genes, respectively. No isolates, however, carried a combination of all three genes. On the contrary, Rios et al. (45) isolated enterohemorrhagic STEC serogroups O26 and O111 from pig intestinal contents, and the strains harbored virulence genes with profiles indicative of potential human pathogens (stx_1, stx_2, *eae*, and *hly* or stx_1, stx_2, and *eae*), suggesting that pigs may be an important animal reservoir for these organisms. Although the presence of STEC in swine has been investigated to some extent to determine if pigs are an important animal reservoir for these organisms, comprehensive surveys of swine for STEC are lacking in the United States.

Unlike *E. coli* O157:H7 strains, which generally are sorbitol and β-glucuronidase negative, the non-O157 STEC strains do not have identifiable biochemical markers to facilitate screening for and identification of these pathogens. Furthermore, since there are no phenotypic markers shared by all non-O157 STEC strains, except for the production of Shiga toxins, that can be utilized to distinguish them from nonpathogenic *E. coli* strains, detection and identification of these pathogens are difficult. Detection of non-O157 STEC serotypes requires testing for the Shiga toxins by immunologic or toxin receptor-mediated enzyme-linked immunosorbent assays, tissue culture assays, and/or PCR or DNA hybridization techniques targeting the Shiga toxin genes (2, 25, 47, 50). Fluorogenic 5′ nuclease

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PCR assays (TaqMan) allow automated PCR amplification, detection, and analysis of pathogens and have been used to detect food-borne pathogens, including STEC, in food, animal feces, and other types of samples (8, 24, 48).

Two main objectives of the National Animal Health Monitoring System (NAHMS) Swine 2000, a national study established by the U.S. Department of Agriculture's (USDA's) Animal and Plant Health Inspection Service, were to measure the degree to which food-borne pathogens are found in finisher pigs and to identify potential control factors to reduce the public health risk from these pathogens (49a). Although human illness associated with swine STEC is uncommon, outbreaks and cases of illness due to different STEC serotypes have occurred in recent years in the United States and worldwide. Thus, the objectives of the present study were (i) to determine the prevalence of STEC in swine in the United States and (ii) to determine the serotypes and types of Shiga toxin genes in the swine STEC isolates.

MATERIALS AND METHODS

Study design and sample collection. A report published by the USDA's Animal and Plant Health Inspection Service and Veterinary Services in August 2001 provides a complete description of the NAHMS 2000 study design (49a). Briefly, fecal samples were obtained from a subset of sites distributed across swine operations from the top 17 pork-producing states, representing 94% of producers in the United States with 100 or more pigs as of 1 December 1999. Samples were collected over a period of 25 weeks during the months from September 2000 to March 2001. Up to 50 fresh fecal samples were obtained from the pen floors of late-finisher pigs (over 20 weeks of age). In addition, up to 10 floor samples were obtained from cull sows (within 10 days of slaughter) when available. Samples were placed in Whirl-Pak bags (Nasco, Modesto, Calif.) and shipped overnight in Styrofoam coolers containing freezer packs to the Richard B. Russell Agricultural Research Center in Athens, Ga. Samples were then processed and shipped overnight in coolers to the Eastern Regional Research Center in Wyndmoor, Pa., for testing. A total of 687 fecal samples were tested for the presence of STEC.

Enrichment of fecal samples. Ten grams of each fecal sample was mixed with 100 ml of tryptic soy broth (TSB; Becton Dickinson, Sparks, Md.) in Bagfilter bags (Spiral Biotech, Inc., Norwood, Mass.) and subjected to enrichment at 37°C for 12 h with shaking at 100 rpm. A subset of samples was plated and processed immediately after enrichment as described below; however, the rest of the samples that could not be processed immediately were frozen at −80°C with 20% glycerol and then thawed and processed later. One-half milliliter of the thawed enrichments was added to 2.5 ml of TSB, which was then incubated at 37°C for 4 h prior to plating onto Luria-Bertani (LB) agar (Becton Dickinson).

DNA extraction and TaqMan PCR. One milliliter of each enrichment sample was subjected to DNA extraction with PrepMan sample preparation reagent (Applied Biosystems, Foster City, Calif.) according to the manufacturer's instructions with the following modifications. Briefly, a 1-ml sample was centrifuged at 16,000 × *g* for 2 min, and the pellet was washed twice with sterile water and then resuspended in 200 μl of PrepMan reagent. The sample was then heated at 100°C for 10 min and centrifuged at 16,000 × *g* for 2 min. The supernatant (100 μl) was collected, and 5 μl of each sample was used for the TaqMan PCR assays. The TaqMan *E. coli* STX1 and STX2 detection kits (Applied Biosystems), targeting the stx_1 and stx_2 genes, respectively, were used according to the manufacturer's instructions to detect the presence of STEC in the fecal samples. The assay was performed in a 96-well format by use of a GeneAmp 9600 PCR system instrument (Applied Biosystems), and PCR products were detected using an ABI Prism 7200 sequence detector with Sequence Detector software (version 1.6.3).

Colony hybridization and confirmation of isolates by multiplex PCR using primers targeting stx_1, stx_2, and stx_{2e}. Diluted portions (100 μl of 1:1,000 and 1:10,000 dilutions) of the enrichment samples that were positive for stx_1 and stx_2 by the TaqMan assays were plated onto LB agar, and the plates were incubated at 37°C for 20 h. Hybond-N+ membranes (Amersham Pharmacia Biotech, Piscataway, N.J.) were marked for orientation, and colony lifts were performed with the plates having ca. 100 to 200 colonies. Following denaturation and neutralization of the membranes, performed according to instructions in the PCR DIG probe synthesis kit user's manual (Roche Diagnostics GmbH, Indianapolis, Ind.), the DNA was cross-linked to the membranes by use of a Stratalinker 2400 instrument (Stratagene, La Jolla, Calif.). The digoxigenin (DIG)-labeled stx_1 and stx_2 DNA probes were prepared with the stx_1 and stx_2 PCR products (see below) by using the PCR DIG probe synthesis kit (Roche Diagnostics GmbH) according to the manufacturer's instructions. The membranes were placed into hybridization bottles and subjected to prehybridization for 1 h, followed by hybridization with a mixture of the two DIG-labeled probes (12 ml of hybridization solution; four membranes/tube) according to the instructions for the DIG nucleic acid detection kit (Roche Diagnostics GmbH). After color development, colonies that aligned with spots on the membrane were picked and tested by multiplex PCR with primers for the stx_1 and stx_2 genes. Up to four colonies were picked and tested per plate. Colonies were suspended in 40 μl of sterile water and heated at 99°C for 10 min in a GeneAmp PCR System 9600 thermal cycler (Applied Biosystems) to lyse the bacteria. Oligonucleotide primer sequences used for amplification of regions in the stx_1 and stx_2 genes, yielding products of 210 and 484 bp, respectively, were as follows: SLT1F, 5′-TGTAACTGGAAAGGTGGAGTATAC A-3′; SLT1R, 5′-GCTATTCTGAGTCAACGAAAAATAAC-3′; SLT2F, 5′-GTT TTTCTTCGGTATCCTATTCC-3′; and SLT2R, 5′-GATGCATCTCTGGTCATT GTATTAC-3′ (34). The PCR reagent system kit (Invitrogen, Carlsbad, Calif.) was employed to prepare the PCR mixture, which consisted of 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 3.0 mM $MgCl_2$, 0.1% Triton X-100, a 400 μM concentration of each of the four deoxynucleotide triphosphates, 2.5 U of *Taq* DNA polymerase, a 0.5 μM concentration of each of the primers, and 5 μl of template DNA. The cycling protocol, using the primers for stx_1 and stx_2, consisted of denaturation at 94°C for 2 min; 35 cycles at 94°C for 20 s, 57°C for 1 min, and 72°C for 1 min; and a final extension at 72°C for 10 min. Primers for amplification of a region of the stx_{2e} gene (a 230-bp product) were VT2e-A and VT2e-B, whose sequences were 5′-CCTTA ACTAAAAGGAATATA-3′ and 5′-CTGGTGGTGTATGATTAATA-3′, respectively (6). The cycling protocol consisted of 94°C for 3 min; 30 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min; and a final extension at 72°C for 10 min. Isolates that produced stx_1, stx_2, or stx_{2e} were stored at −70°C in TSB with 20% glycerol.

Serotyping of isolates. Isolates were sent to the Gastroenteric Disease Center at The Pennsylvania State University (University Park, Pa.) for serotyping.

RESULTS AND DISCUSSION

Prevalence of STEC in swine fecal samples. Out of a total of 687 swine fecal samples tested for the presence of stx_1 and stx_2 by the TaqMan STX1 and STX2 PCR assays, 484 (70%) samples were positive for stx_1, stx_2, or both toxin genes. Experiments using fecal samples artificially inoculated with *E. coli* O157:H7 strains that harbored both stx_1 and stx_2 (a mixture of strains 933 and 380-94) showed that the detection limit of the TaqMan assays for both stx_1 and stx_2 was ca. 10^3 CFU/10 g of feces. Up to four colonies were picked from plates that were positive by DNA hybridization (from 196 different fecal samples). Thus, isolates were obtained from ca. 40% (196 of 484) of the stx_1- or stx_2-positive samples. This result is in agreement with the results of Botteldoorn et al. (10), who were able to isolate a STEC strain in only one-third of the PCR-positive samples from pigs. These authors suggested that this rate of isolation was due the presence of both high levels of background microflora and low levels of STEC in the pig fecal samples. In the present study, a nonselective enrichment medium (TSB) was used to allow recovery of stressed or injured STEC in the fecal samples. The use of a selective agar rather than LB agar might have resulted in higher recovery rates from samples with low levels of STEC, since selective agar may have prevented the growth of a portion of the non-STEC bacteria; thus, lower dilutions of the enrichments could possibly have been plated on a selective agar. Furthermore, a nonselective enrichment in TSB followed by a selective enrichment could also have been performed, and additional STEC isolates may also have been obtained if colony lifts and hybridizations had

TABLE 1. Detection of Shiga toxin genes by PCR in swine fecal samples and characterization of the swine STEC isolates

Kit[a]	State	Date (mo/day/yr)	TaqMan PCR results			PCR results				*E. coli* serotyping results[d]	
			Total no. of samples tested	No. of samples positive for:		Total no. of isolates[b] tested	No. of isolates[c] positive for:			O antigen	H antigen
				stx$_1$	*stx*$_2$		*stx*$_1$	*stx*$_2$	*stx*$_{2e}$		
6	Ill.	9/6/2000	60	17	12	3	0	0	3	O8, O120	H(−), H9
2	Nebr.	9/18/2000	50	37	41	18	0	0	18	O120, O8, O121, O65, O2, O78	H44, H(−)
30	Iowa	9/18/2000	10	8	2	3	1	0	2	O120, O91	H(−)
35	Minn.	9/19/2000	50	7	9	31	0	4	27	O(−), O7, O8, O8w, O9w, O11w, O20, OX10, O96	H(−), H(+), H3, H5, H9, H19, H25, H30, H42
14[e]	Mich.	9/20/2000	28	15	12	7	2	0	5	O(−), O8, O20	H19, H42, H51
34	Minn.	9/20/2000	24	17	22	14	0	0	14	O8	H(−), H9, H19, H20
20[e]	Iowa	10/2/2000	19	15	15	3	1	2	0	O159, O152	H(−), H4w
21	Iowa	10/2/2000	21	18	14	10	1	2	7	O(−), O15, O8, O100w, O121, O159, O171, O69, O5	H(−), H9, H10, H17, H26, H30, H34
33	Ind.	10/2/2000	18	17	13	4	0	0	4	O8, O(−)	H19, H20, H42
37	S.D.	10/2/2000	9	9	9	5	5	0	0	O91	H(−), H12
61[e]	Nebr.	10/16/2000	45	31	41	33	1	2	30	O(−), O8, O8w, O9, O20, O91, O100, O100w	H(−), H9, H17, H30, H34, H44
26	Iowa	10/17/2000	39	8	8	3	3	0	0	O(−), O163, OX18	H(−), H51, H24, H56
78	Iowa	10/30/2000	25	13	19	12	4	0	8	O(−), O91, O8	H(−)
52	Ark.	10/30/2000	25	19	24	0	0	0	0		
44[e]	Minn.	10/31/2000	20	9	19	16	1	0	15	O(−), O8, O160, O20	H(−)
59	N.C.	10/31/2000	20	0	17	3	0	0	3	O(−), O100w	H30, H(−)
74	Ind.	10/31/2000	20	4	20	5	0	0	5	O(−), O8	H(−)
84	N.C.	11/28/2000	32	0	31	6	0	0	6	O121, O159	H(−), H10
87	Iowa	11/27/2000	18	0	7	4	0	0	4	O(−), O8, O120	H(−), H9
142	Iowa	2/5/2001	37	33	10	3	3	0	0	O163	H(−), H41, H51
132	Kans.	2/6/2001	20	20	5	1	0	1	0	O159	H4
137	Minn.	2/6/2001	25	23	15	10	0	0	10	O(−), O8, O68, O101	H(−), H20, H37
66	Iowa	10/16/2000	5	5	5	3	3	0	0	A, O91	H(−), H14
53[e]	Wis.	10/16/2000	7	1	6	9	2	1	6	O(−), O8, OX18, O57w, O91, O100, O120	H14, H17, H23, H30
150	Iowa	3/20/2001	16	14	16	4	0	1	3	O8, O57w, O121	H(−)
151	Iowa	3/20/2001	14	7	14	0	0	0	0		
152	Iowa	3/20/2001	15	12	15	3	0	1	2	O(−), O8, O20	H(−), H4w
102	Iowa	3/19/2001	15	11	15	6	2	0	4	O8, OX18, O121, O163	H(−)
Total			687	370	436	219	29	14	176		

[a] Samples collected from one location on one day comprised one kit.
[b] Total number of isolates obtained from different *stx*$_1$- and/or *stx*$_2$-positive fecal samples from each kit.
[c] Number of isolates shown to possess *stx*$_1$, *stx*$_2$, or *stx*$_{2e}$ by PCR targeting those genes.
[d] (−), no reaction with standard antisera; (+), *fliC* gene was amplified by PCR, but no agglutination reaction occurred with any of the H typing antisera; A, autoagglutination; w, weak reaction.
[e] Kit contained samples that had an isolate harboring *stx*$_1$ and an isolate harboring *stx*$_2$ or *stx*$_{2e}$.

been performed on several plates obtained from the same enrichment sample. Analysis of colonies from the 196 DNA hybridization-positive samples showed that 23 of the 196 samples from which STEC strains were isolated contained at least two different strains of different serogroups. Thus, the data in Table 1 show results for 219 STEC isolates, i.e., one isolate from each of 173 fecal samples and two isolates from each of 23 samples.

Several studies have examined the prevalence of STEC in swine. Parma et al. (42) isolated enterotoxigenic *E. coli* (ETEC) and VTEC from rectal swabs of healthy pigs and pigs with diarrhea over a period of 5 years in Argentina. The ETEC and VTEC strains were isolated from 5% of the rectal swabs obtained from 223 pigs. Isolated colonies were tested by PCR for Shiga toxin genes and genes associated with ETEC. The isolates that harbored *stx*$_{2e}$, all recovered from pigs that showed delayed growth or that had diarrhea or edema disease, belonged to serogroup O8, O138, or O139 or were untypeable. A number of isolates that possessed *stx*$_{2e}$ also had additional virulence genes, including the LTI, STIa, and STb genes. Botteldoorn et al. (10) isolated *E. coli* strains with the *stx*$_{2e}$ variant from 31% of pig rectal samples, but no strains had *stx*$_1$. Additional studies have shown that the incidence of STEC in pigs may be as high as 69% (9, 45). The strains isolated from pigs had toxin profiles similar to those of strains from humans and food (45) and included strains that possessed the *stx*$_1$ and *stx*$_2$ genes; however, it was not determined if the strains that harbored *stx*$_2$ actually possessed the *stx*$_{2e}$ variant. A study conducted in northern Italy showed that STEC strains were present in 7.8% (19 of 242) of fecal samples obtained from healthy pigs (12). Fecal samples were enriched in Trypticase soy broth overnight, and the supernatants were tested by the Vero cell assay for the presence of Shiga toxins. Five isolates belonging to serogroups O101 and O8 were recovered from

Vero cell assay-positive samples. These studies show that the incidence of STEC in swine varies and that the variation in methods employed for detection and isolation of STEC may be a factor contributing to differences among measurements of prevalence. Furthermore, the prevalence of *E. coli* O157:H7 and non-O157 STEC in animals may be lower in the winter months. Thus, since the fecal samples tested in the present study were collected during the months of September through March, the prevalence of STEC may have been somewhat lower than if samples had been collected during the summer months.

STEC serogroups isolated from swine fecal samples. A number of STEC serogroups have been isolated from pigs, including O2, O5, O7, O8, O9, O15, O65, O91, O101, O120, O121, and O163 (12, 15, 22, 23, 29, 36). All of these serogroups and several others were isolated from pig fecal samples examined in the present study (Tables 1 and 2). Studies have shown that STEC can be isolated, often at similar frequencies, from healthy weaned pigs and pigs with diarrhea or edema disease (15, 22, 23, 36, 41). Feces tested in the present study were obtained from cull sows and pigs that were apparently healthy and that did not have diarrhea or symptoms of edema disease. The most prevalent *E. coli* serogroups isolated from pigs with edema disease and postweaning diarrhea are O138, O139, O141, and O149 (21, 36, 40), which were not isolated from swine feces examined in the present study (Tables 1 and 2). With the exception of several "rough " STEC strains, all isolates obtained from pigs with edema disease or diarrhea in Denmark and several other European countries belonged to serogroup O139 (1). Interestingly, all of the *E. coli* O139 isolates that harbored stx_{2e} were grouped into one cluster by pulsed-field gel electrophoresis, indicating the potential spread of one clone among the pigs. A similar study also showed that *E. coli* O139 was the predominant STEC strain isolated from pigs with edema disease (14).

Shiga toxin genes in swine STEC. In the present study, multiple isolates from a single positive sample typically displayed the same phenotype; however, six fecal samples contained a strain that harbored the stx_1 gene in addition to a strain that harbored stx_2 or stx_{2e}. The samples were the following: (i) kit 14, sample 35, *E. coli* O8:H19 (stx_{2e}) and O20:H19 (stx_1); (ii) kit 20, sample 39, *E. coli* O159:H(−) (stx_2) and O152:H(−) (stx_1); (iii) kit 44, sample 37, *E. coli* O8:H(−) (stx_{2e}) and O160:H(−) (stx_1); (iv) kit 53, sample 2, *E. coli* O8:H17 (stx_{2e}) and OX18:H23 (stx_1); (v) kit 53, sample 6, *E. coli* O(−):H30 (stx_{2e}) and O91:H14 (stx_1); and (vi) kit 61, sample 45, *E. coli* O100:H30 (stx_{2e}) and O91:H(−) (stx_1). Strains of *E. coli* serogroup O91, possessing different H types, have been isolated from healthy pigs and pigs with diarrhea (5, 15, 23). Many of the *E. coli* O91 strains produced stx_1. In the present study, 16 isolates belonged to serogroup O91 (Table 2). Fourteen isolates [belonging to serogroups O91:H(−), O91:H12, and O91:H14] harbored stx_1, and two isolates (serogroup O91:H44) harbored stx_{2e}. *E. coli* O157:H7 has been isolated from swine feces and intact-colon fecal samples from several countries, including the United States, Japan, and Norway (17, 26, 37); however, *E. coli* O157:H7 was not isolated from swine feces examined in the present study, nor was this serotype isolated in a separate project, also part of the NAHMS Swine 2000 study, that specifically targeted *E. coli*

TABLE 2. *E. coli* serotypes isolated from swine fecal samples possessing the stx_1, stx_2, or stx_{2e} genes

Serotype(s) of isolates	No. of isolates possessing[a]:		
	stx_1	stx_2	stx_{2e}
O(−):H(−)			10
O(−):H(4)			1
O(−):H9			1
O(−):H20			1
O(−):H30		1	11
O(−):H34			1
O(−):H42			2
O(−):H51	1		
O(−):H51, H24[b]	1		
O2:H44			1
O5:H17			1
O7:H(+)[c]		1	
O8:H(−)		2	35
O8:H3		1	
O8:H9		1	20
O8:H17			2
O8:H19			8
O8:H20			3
O8:H20, H37[b]			3
O9:H9			1
O9:H17			2
O9:H19			1
O11:H25			1
O15:H(−)	1		
O20:H(−)			3
O20:H19	1		
O20:H30			5
O20:H42			3
O57:H(−)			1
O57:H14			1
O65:H(−)			1
O68:H(−)			1
O69:H26			2
O78:H(−)			1
O91:H(−)	10		
O91:H12	2		
O91:H14	2		
O91:H44			2
O96:H5		1	
O100:H(−)			1
O100:H30		1	20
O101:H(−)			4
O120:H(−)			15
O120:H30		1	
O121:H(−)		1	2
O121:H10			7
O152:H(−)	1		
O159:H(−)		1	1
O159:H4		2	
O159:H34			1
O160:H(−)	1		
O163:H(−)	4		
O163:H41, H51[b]	1		
OX10:H(−)		1	
OX18:H(−)	1		
OX18:H23, H24[b]	1		
OX18:H24, H56[b]	1		
A:H(−)	1		
Total	29	14	176

[a] Number of strains of the *E. coli* serotype in which a specific toxin gene was found.

[b] Agglutination occurred with two different H-antisera, as indicated.

[c] (+), *fliC* gene amplified by PCR, but no agglutination reactions observed with any of the H typing antisera.

O157:H7 (I. Feder, J. T. Gray, R. A. Pearce, E. Bush, P. Fratamico, F. M. Wallace, A. Porto, P. Fedorka-Cray, R. Perrine, R. Dudley, and J. B. Luchansky, Abstr. Int. Assoc. Food Prot. Annu. Meet., abstr. P120, 2002).

Out of a total of 687 fecal samples tested, 54% (370 of 687), 64% (436 of 687), and 38% (261 of 687) were positive for stx_1, stx_2, and both toxin genes, respectively, by PCR. Twenty-nine (13%), 14 (6%), and 176 (80%) of the 219 isolates obtained from the PCR-positive samples possessed the stx_1, stx_2, and stx_{2e} genes, respectively (Table 2). The TaqMan STX1 and STX2 PCR assays are rapid, sensitive, and able to differentiate between stx_1 and stx_2; however, the assays cannot differentiate between stx_2 and stx_{2e}. Primers targeting stx_{2e} were subsequently used to determine if strains harboring stx_2 (a 484-bp product) possessed the stx_{2e} variant (a 230-bp product), which is found in a large proportion of STEC strains from healthy pigs, as well as from pigs with postweaning diarrhea and edema disease (15, 40, 41). In the present study, a large proportion of the swine STEC strains harbored stx_{2e}. Although Stx_{2e}-producing STEC strains have caused diarrhea and HUS in humans (35, 44, 49), swine STEC strains (harboring stx_{2e}) are more likely to cause disease in swine. Osek (41) isolated 76 and 2 STEC strains from pigs with diarrhea and healthy pigs, respectively; all strains harbored the stx_{2e} variant. A study in Brazil found that 69% (99 of 144) of the *E. coli* strains isolated from pigs with edema disease possessed stx_{2e} (14). Apparently, swine-infecting *E. coli* strains that produce Shiga toxins compose a heterogeneous group. Many also possess genes encoding the F18 fimbriae, hemolysins, stable toxin or labile toxin (14, 22, 36). Research examining the presence of these and other genes associated with virulence in the swine STEC isolated in the present study is in progress.

Swine STEC serogroups that are potential human pathogens. Twenty-nine and 14 strains isolated from the swine feces possessed the stx_1 and stx_2 genes, respectively. Thus, these strains are potential human pathogens (Table 2). Furthermore, most of the STEC serogroups that were isolated have been associated with human illnesses (15, 16, 20, 28, 33, 43, 49). For example, *E. coli* strains of serotypes O91:H(−), O91:H10, O91:H21, and O91:H40 have been associated with cases of bloody diarrhea and HUS (7, 28, 43). *E. coli* O121 has also been reported in cases of human illness, and *E. coli* O121:H19 was associated with an outbreak of HUS at a lake in Connecticut in 1999 (20, 31). *E. coli* O101 strains [H types NM, H(−), and H9] have caused bloody diarrhea and HUS (28, 44, 49). Interestingly, both the *E. coli* O101:H(−) and O101:H9 strains isolated in these studies possessed the stx_{2e} variant gene. To determine the clonal relatedness of human and porcine *E. coli* O101 strains, Franke et al. (19) sequenced the stx_{2e} gene of one human *E. coli* O101 strain (isolated from a patient with diarrhea) and four porcine *E. coli* O101 strains and performed virulence factor analysis and DNA fingerprinting with repetitive-element sequence-based PCR. The DNA sequences of the stx_{2e} genes in these strains showed high identity to those in the classical STEC O139 strains that cause edema disease in swine. There was a high degree of genetic relatedness among the one human and four porcine O101 strains based on DNA fingerprinting, and the patterns of the five O101 strains did not show relatedness to those of several other STEC serogroups. However, virulence factors typically found in porcine STEC (i.e., stable toxin, labile toxin, and F107 fimbriae) and human STEC (i.e., *eaeA* and enterohemorragic *E. coli* hemolysin) were not found in the *E. coli* O101 strains. This result indicated that the human Stx2e-producing *E. coli* O101 strain, lacking typical virulence factors found in human STEC, caused illness via a different pathogenic mechanism. Furthermore, Franke et al. suggested that PCR assays targeting the stx_{2e} variant be employed as diagnostic tools to detect and identify potential human pathogens harboring this gene.

Conclusions. The results of this study indicate that swine may be a potential reservoir of STEC strains that cause human illness. In fact, 13.2 and 6.4% of the studied strains possessed the stx_1 and stx_2 genes, respectively; these genes are typically found in human STEC strains. Moreover, the majority (80%) of the STEC strains isolated from the swine feces harbored the porcine STEC stx_{2e} variant. The extent to which swine play a role in the epidemiology of human infection, however, needs further investigation. Our studies provide information for the establishment of a database of STEC strains harbored by pigs in the United States, which is necessary for the implementation of measures for disease prevention. This database would require periodic evaluation and frequent updating when new swine STEC serotypes are discovered.

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